PATENT COOPERATION TREATY

From the INTERNATIONAL BUREAU

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

United States Patent and Trademark Office
(Box PCT)
Crystal Plaza 2
Washington, DC 20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 26 January 1999 (26.01.99)	
International application No. PCT/NL98/00325	**Applicant's or agent's file reference** PCT 0702
International filing date (day/month/year) 03 June 1998 (03.06.98)	**Priority date** (day/month/year) 04 June 1997 (04.06.97)
Applicant BAKKER, Egbert et al	

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

04 January 1999 (04.01.99)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO **34, chemin des Colombettes** **1211 Geneva 20, Switzerland**	Authorized officer Nicola Wolff
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

INTERNATIONAL SEARCH REPORT

Interna[illegible]l Application No
PCT/NL 98/00325

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PUGET ET AL.: "A 1-kb Alu mediated germ line deletion removing BRCA1 exon 17" CANCER RESEARCH., vol. 57, March 1997, pages 828-831, XP002057724 MD US see the whole document ---	1-6, 11-14
X	EP 0 705 903 A (MYRIAD GENETICS INC ;RECH DU CHUL CENTRE (CA); CANCER INST (JP)) 10 April 1996 see the whole document ---	1-6,14
X	US 5 622 829 A (KING MARY-CLAIRE ET AL) 22 April 1997 see column 4, line 15 - line 30; table 1 ---	1-6,14
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 September 1998	07/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Molina Galan, E

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 699 754 A (UNIV UTAH RES FOUND ;US GOVERNMENT (US); MYRIAD GENETICS INC (US)) 6 March 1996 see claims; example 9; table 11 ---	1-3,6,14
X	WO 94 21791 A (BERGMANN JOHANNA EUGENIE ;PREDDIE RICK ENRIQUE (CA)) 29 September 1994 see page 15 ---	1
A	SMITH ET AL.: "Complete genomic sequence and analysis of 117 kb of human DNA containing the gene BRCA1" GENOME RESEARCH., vol. 6, 1996, pages 1029-1049, XP002057725 ING HARBOR LABORATORY PRESS US cited in the application see the whole document ---	1-14
A	COUCH ET AL.: "Mutations and polymorphisms in the familial early onset breast cancer (BRCA1) gene" HUMAN MUTATION, vol. 8, 1996, pages 8-18, XP002057726 cited in the application see the whole document ---	1-14
A	RÜDIGER: "One short well conserved region of Alu sequences is involved in human rearrangements and has homology with prokaryotic chi" NUCLEIC ACIDS RESEARCH, vol. 23, no. 2, 1996, pages 256-260, XP002057727 OXFORD GB cited.in the application see the whole document ---	11,12
P,X	DATABASE MEDLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US PETRIJ-BOSCH A ET AL: "BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients 'published erratum appears in Nat Genet 1997 Dec;17(4):503!." XP002078428 see abstract & NATURE GENETICS, (1997 NOV) 17 (3) 341-5. JOURNAL CODE: BRO. ISSN: 1061-4036., United States --- -/--	1-14

INTERNATIONAL SEARCH REPORT

Intern ıal Application No
PCT/NL 98/00325

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 5 756 294 A (WHITE MARGA B ET AL) 26 May 1998 see the whole document	1-6

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr. ial Application No
PCT/NL 98/00325

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0705903 A	10-04-1996	US 5693473 A	02-12-1997
		US 5709999 A	20-01-1998
		AU 691958 B	28-05-1998
		AU 3242895 A	07-03-1996
		AU 686004 B	29-01-1998
		AU 3321295 A	07-03-1996
		AU 691331 B	14-05-1998
		AU 3321695 A	07-03-1996
		CA 2196790 A	22-02-1996
		CA 2196795 A	22-02-1996
		CA 2196797 A	22-02-1996
		CN 1159829 A	17-09-1997
		CN 1172502 A	04-02-1998
		EP 0705902 A	10-04-1996
		EP 0699754 A	06-03-1996
		FI 970513 A	07-04-1997
		FI 970514 A	07-04-1997
		FI 970515 A	07-04-1997
		JP 10505742 T	09-06-1998
		NO 970624 A	14-04-1997
		NO 970625 A	14-04-1997
		NO 970626 A	14-04-1997
		WO 9605306 A	22-02-1996
		WO 9605307 A	22-02-1996
		WO 9605308 A	22-02-1996
		US 5747282 A	05-05-1998
		US 5710001 A	20-01-1998
		US 5753441 A	19-05-1998
US 5622829 A	22-04-1997	AU 5566896 A	07-11-1996
		CA 2217668 A	24-10-1996
		EP 0821733 A	04-02-1998
		WO 9633271 A	24-10-1996
EP 0699754 A	06-03-1996	US 5747282 A	05-05-1998
		US 5710001 A	20-01-1998
		AU 691958 B	28-05-1998
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		AU 686004 B	29-01-1998
		AU 3321295 A	07-03-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern ial Application No
PCT/NL 98/00325

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EP 0699754 A		AU 691331 B	14-05-1998
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		WO 9605308 A	22-02-1996
		US 5693473 A	02-12-1997
		US 5709999 A	20-01-1998
		US 5753441 A	19-05-1998
WO 9421791 A	29-09-1994	NONE	
US 5756294 A	26-05-1998	NONE	

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	**FOR FURTHER ACTION**	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PCT 0702		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/NL 98/ 00325	03/06/1998	04/06/1997
Applicant		
RIJKSUNIVERSITEIT TE LEIDEN ..et al		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. [ ] **Certain claims were found unsearchable** (see Box I).

2. [ ] **Unity of invention is lacking** (see Box II).

3. [ ] The international application contains disclosure of a **nucleotide and/or amino acid sequence listing** and the international search was carried out on the basis of the sequence listing
   - [ ] filed with the international application.
   - [ ] furnished by the applicant separately from the international application,
     - [ ] but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
   - [ ] Transcribed by this Authority

4. With regard to the **title,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**
   - [X] the text is approved as submitted by the applicant
   - [ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this International Search Report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is:

   Figure No. _____
   - [ ] as suggested by the applicant.
   - [ ] because the applicant failed to suggest a figure.
   - [ ] because this figure better characterizes the invention.
   - [X] None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 98/00325

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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X	US 5 622 829 A (KING MARY-CLAIRE ET AL) 22 April 1997 see column 4, line 15 - line 30; table 1 ---	1-6,14
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
23 September 1998	07/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Molina Galan, E

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 699 754 A (UNIV UTAH RES FOUND ;US GOVERNMENT (US); MYRIAD GENETICS INC (US)) 6 March 1996 see claims; example 9; table 11 ---	1-3,6,14
X	WO 94 21791 A (BERGMANN JOHANNA EUGENIE ;PREDDIE RICK ENRIQUE (CA)) 29 September 1994 see page 15 ---	1
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A	COUCH ET AL.: "Mutations and polymorphisms in the familial early onset breast cancer (BRCA1) gene" HUMAN MUTATION, vol. 8, 1996, pages 8-18, XP002057726 cited in the application see the whole document ---	1-14
A	RÜDIGER: "One short well conserved region of Alu sequences is involved in human rearrangements and has homology with prokaryotic chi" NUCLEIC ACIDS RESEARCH, vol. 23, no. 2, 1996, pages 256-260, XP002057727 OXFORD GB cited in the application see the whole document ---	11,12
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1

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category ³	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 5 756 294 A (WHITE MARGA B ET AL) 26 May 1998 see the whole document	1-6

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 98/00325

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0705903 A	10-04-1996	US 5693473 A	02-12-1997
		US 5709999 A	20-01-1998
		AU 691958 B	28-05-1998
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		CA 2196797 A	22-02-1996
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		CN 1172502 A	04-02-1998
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		EP 0699754 A	06-03-1996
		FI 970513 A	07-04-1997
		FI 970514 A	07-04-1997
		FI 970515 A	07-04-1997
		JP 10505742 T	09-06-1998
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		WO 9633271 A	24-10-1996
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		AU 691958 B	28-05-1998
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		AU 686004 B	29-01-1998
		AU 3321295 A	07-03-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/NL 98/00325

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EP 0699754 A		AU 691331 B	14-05-1998
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		NO 970624 A	14-04-1997
		NO 970625 A	14-04-1997
		NO 970626 A	14-04-1997
		WO 9605306 A	22-02-1996
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		WO 9605308 A	22-02-1996
		US 5693473 A	02-12-1997
		US 5709999 A	20-01-1998
		US 5753441 A	19-05-1998
WO 9421791 A	29-09-1994	NONE	
US 5756294 A	26-05-1998	NONE	

PATENT COOPERATION TREATY

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

OTTEVANGERS,S.U.
VEREENIGDE OCTROOIBUREAUX
Nieuwe Parklaan 97
NL-2587 BN The Hague
PAYS-BAS

PCT

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT
(PCT Rule 71.1)

Date of mailing *(day/month/year)*	- 8. 09. 99
Applicant's or agent's file reference P22163 PC00	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL98/00325	03/06/1998	04/06/1997

Applicant
RIJKSUNIVERSITEIT TE LEIDEN ..et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

4. **REMINDER**

The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/

European Patent Office - P.B. 5818 Patentlaan 2
NL-2280 HV Rijswijk - Pays Bas
Tel. (+31-70) 340-2040 Tx: 31 651 epo nl
Fax: (+31-70) 340-3016

Authorized officer

Reisinger, E

Tel.(+31-70)-340-2974

PATENT COOPERATION TREATY

ONTVANGEN
13 SEP 1999
AMERSFOORT
PCT

Ren

From the
INTERNATIONAL PRELIMINARY EXAMINING AUTHORITY

To:

OTTEVANGERS,S.U.
VEREENIGDE OCTROOIBUREAUX
Nieuwe Parklaan 97
NL-2587 BN The Hague
PAYS-BAS

4-12-99

Kopie in/naar | Beantwoord voorl. | Bericht gezonden aan

NOTIFICATION OF TRANSMITTAL OF THE INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Rule 71.1)

	Date of mailing *(day/month/year)* - 8. 09. 99
Applicant's or agent's file reference P22163 PC00	**IMPORTANT NOTIFICATION**

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL98/00325	03/06/1998	04/06/1997

Applicant
RIJKSUNIVERSITEIT TE LEIDEN ..et al

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.

2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.

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For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

Name and mailing address of the IPEA/	Authorized officer
European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. (+31-70) 340-2040 Tx: 31 651 epo nl Fax: (+31-70) 340-3016	Reisinger, E Tel.(+31-70)-340-2974

Form PCT/IPEA/416 (July 1992)

2.3 INVENTIVE STEP (Art. 33(3) PCT)

2.3.1 Detection of a deletion mutation with a probe complementary to sequences on both sides of the deletion is only one of the routine options from which the person skilled in the art would choose in the absence of inventive skills and claims 4, 12 and 13 can not be considered to involve an inventive step in the sense of Article 33(3) PCT.

2.3.2 No cited prior art however teaches or suggests the presence of deletions related to breast cancer in exons 13 or 22 of the BRCA1 gene and these features provide a basis for new and inventive subject matter.

2.3.3 The present application does not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of claims 1-6 and 11-14 does not involve an inventive step (Rule 65(1)(2) PCT).

VII. Certain defects (Continuation)

1 Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in documents D1-D4 is not mentioned in the description, nor are these documents identified therein.

VIII. Certain Observations (Continuation)

1 It is not clear to which numbering system the nucleotides of claim 8 refer to. Accordingly, the claim lacks clarity as required by Art. 6 PCT.

PATENT COOPERATION TREATY

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PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
P22163 PC00		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/NL98/00325	03/06/1998	04/06/1997
International Patent Classification (IPC) or national classification and IPC C12Q1/68		
Applicant RIJKSUNIVERSITEIT TE LEIDEN ..et al		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☐ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04/01/1999	- 8. 09. 99
Name and mailing address of the international preliminary examining authority: European Patent Office - P.B. 5818 Patentlaan 2 NL-2280 HV Rijswijk - Pays Bas Tel. (+31-70) 340-2040 Tx: 31 651 epo nl Fax: (+31-70) 340-3016	Authorized officer Molina Galan, E Telephone No. (+31-70)-340

Form PCT/IPEA/409 (cover sheet) (January 1994)

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/NL98/00325

I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

Description, pages:

1-22 as originally filed

Claims, No.:

1-14 as originally filed

Drawings, sheets:

1/4-4/4 as originally filed

2. The amendments have resulted in the cancellation of:

☐ the description, pages:
☐ the claims, Nos.:
☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	4, 7-10, 12 and 13
	No:	Claims	1-3, 5, 6, 11 and 14
Inventive step (IS)	Yes:	Claims	7-10
	No:	Claims	1-6 and 11-14
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	

2. Citations and explanations

see separate sheet

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

V. Reasoned statement (Continuation)

2.1 CITATIONS

Reference is made to the following documents:

D1: EP-A-699 754, Myriad Genetics Inc.
D2: US-A-5 622 829, King et al.
D3: WO-A-9421791, Bergmann et al.
D4: Cancer Research, 57, 1.3.97, 828-831, Puget et al.

2.2 NOVELTY (Art. 33(2) PCT)

2.2.1 D1 discloses methods for diagnosing the predisposition to breast cancer by detecting deletions in the BRCA1 gene using (allele specific) probes (and amplification, cf claims and example 9). Some of the deletions cause frameshift mutations (cf tables 11 and 12).

2.2.2 D2 discloses deletion mutations in BRCA1 related to breast cancer (cf table 1) and methods for its detection by primer/probes flanking the sides of the deletion (cf column 4, lines 15-30).

2.2.3 D3 discloses the relationship between BRCA locus deletions and breast cancer (cf page 15, first and last paragraph).

2.2.4 D4 discloses the relationship between a large Alu mediated deletion involving exon 17 in BRCA1 and breast cancer (cf abstract and discussion).

2.2.5 The present application does not satisfy the criterion set forth in Article 33(2) PCT because the subject-matter of claims 1-3, 5, 6, 11 and 14 is not new in respect of prior art as defined in the regulations (Rule 64(1)-(3) PCT).

2.3 INVENTIVE STEP (Art. 33(3) PCT)

2.3.1 Detection of a deletion mutation with a probe complementary to sequences on both sides of the deletion is only one of the routine options from which the person skilled in the art would choose in the absence of inventive skills and claims 4, 12 and 13 can not be considered to involve an inventive step in the sense of Article 33(3) PCT.

2.3.2 No cited prior art however teaches or suggests the presence of deletions related to breast cancer in exons 13 or 22 of the BRCA1 gene and these features provide a basis for new and inventive subject matter.

2.3.3 The present application does not satisfy the criterion set forth in Article 33(3) PCT and the subject-matter of claims 1-6 and 11-14 does not involve an inventive step (Rule 65(1)(2) PCT).

VII. Certain defects (Continuation)

1 Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in documents D1-D4 is not mentioned in the description, nor are these documents identified therein.

VIII. Certain Observations (Continuation)

1 It is not clear to which numbering system the nucleotides of claim 8 refer to. Accordingly, the claim lacks clarity as required by Art. 6 PCT.

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(54) Title: A DIAGNOSTIC TEST KIT FOR DETERMINING A PREDISPOSITION FOR BREAST AND OVARIAN CANCER, MATERIALS AND METHODS FOR SUCH DETERMINATION

(57) Abstract

The present invention relates generally to the field of human genetics, and more specifically to the detection of a specific type of germline mutations in the BRCA1 gene, which will predispose to breast and ovarian cancer. In addition, the invention relates to the molecular genetic mechanism that may have mediated the genesis of these mutations, in particular the role of Alu repetitive DNA elements present in the intronic regions of BRCA1. The invention further relates to somatic mutations of this type in the BRCA1 gene in human breast and ovarian cancer, and their use in the diagnosis and prognosis of human breast and ovarian cancer. The invention more particularly relates to the screening of this type of BRCA1 mutations in human genomic DNA, which are useful for the diagnosis of inherited predisposition to breast and ovarian cancer.

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Title: A diagnostic test kit for determining a predisposition for breast and ovarian cancer, materials and methods for such determination.

The present invention relates generally to the field of human genetics. In particular the invention relates to methods and means (diagnostic test kits) for studying the predisposition for certain types of cancers often having a hereditary component and more specifically to the detection of a specific type of germline mutations in genes involved or associated with certain types of hereditary cancers, in particular the (human) BRCA1 gene, which will predispose to breast and ovarian cancer. In addition, the invention reveals a molecular genetic mechanism that may have mediated the genesis of these mutations, in particular the role of Alu repetitive DNA elements present in the intronic regions of BRCA1. The invention further relates to somatic mutations of this type in the BRCA1 gene in human breast and ovarian cancer, and their use in the diagnosis and prognosis of human breast and ovarian cancer.

The invention also relates to the screening of this type of BRCA1 mutations in human genomic DNA, as part of clinical protocols for the diagnosis of inherited predisposition to breast and ovarian cancer.

Background of the invention

Breast cancer is the most common malignancy among women in the Netherlands, with a cumulative risk by age 85 of one in 11. The strongest epidemiological risk factor for the disease is a positive family history.
Depending on the age of diagnosis and occurrence of bilateral disease in the index case, first degree relatives may have a relative risk of up to 10 for developing breast cancer. In the US population, 6 to 19% of women with breast cancer have at least one affected relative at the time of diagnosis [1],

but not all of them are expected to be true genetic cases as the high incidence of breast cancer in the general population will inevitably cause some coincidental familial clustering. In an attempt to stratify the two classes, criteria to define truly inherited breast cancer have been proposed [2]. Such cases are characterized by early age of onset (premenopausal), excess of bilaterality, and clear paternal or maternal transmission with an autosomal dominant mode of inheritance. Approximately 5% of all cases comply with these criteria, while another 13% are classified as familial clustering[3]. Since early age of onset appears to be a hallmark of hereditary breast cancer, one may suspect that among these cases the genetic component is much higher. Indeed, up to 36% of cases diagnosed under the age of 30 are expected to be genetic [4]. No such data are available for the Dutch situation, and little or none of this has been confirmed at the molecular genetic level.

Linkage analysis of early-onset breast cancer families localized BRCA1 to the long arm of chromosome 17 [5]. Further analyses of additional families revealed that women inheriting a mutant allele of BRCA1 are also at increased risk for ovarian cancer [6,7]. Overall, approximately 45% of all families in which breast cancer is the predominant malignancy are due to BRCA1, as are over 80% of all families with both breast and ovarian cancer [6,8]. Female mutation carriers have been estimated to have an 87% risk to develop breast cancer before the age of 70, and 63% risk to develop ovarian cancer before that age [7]. However, significant evidence for ovarian cancer risk heterogeneity was obtained, indicating the existence of at least two classes of BRCA1 mutations; one conferring a high risk to both breast and ovarian cancer, and one conferring a high risk to breast cancer, but only a moderate risk to ovarian cancer, with the former comprising approximately 26% of all BRCA1 mutations [9]. The gene frequency of BRCA1 has been estimated to be 1 in 833 women [10]. This would imply that 1.7% of all breast

cancer patients diagnosed between age 20 and 70 are carrier of such a mutation.

The gene structure of BRCA1 was found to consist of 22 coding exons spanning >80 kb of genomic DNA [11], and encoding a 7.8 kb transcript [12]. An unusually large exon 11 of 3.4 kb comprises 61% of the coding domain. Over 900 mutations in BRCA1 have been published to date and compiled into an electronically accessible database [13]. Several characteristics stand out [14]. First, they are nearly ubiquitously distributed over the gene. Second, >85% of the mutations in the database lead to premature termination of protein translation. These include basepair substitutions leading to a stop codon, small insertions and deletions (of 1 to 40 basepairs) leading to a frame-shift, or splice-site mutations leading to deletions of complete exons and frame shifts. That these changes presumably inactivate gene function is supported by the finding that the great majority of breast and ovarian tumours that develop in BRCA1 mutation carriers show loss of the wildtype allele [15]. The relevance in terms of cancer predisposition of the missense mutations remains a matter of debate. Some of them appear rare polymorphic variants, as they are also observed in control samples. Others seem to affect critical residues, such as the cysteines in the amino-terminal ring finger domain [12], which are conserved in the mouse Brca1 sequence [16]. Third, a number of mutations have been found repeatedly, reducing the number of distinct mutations to about 150. Two of these, the 185delAG mutation and the 5382insC mutation, each represent approximately 11% of all mutations thus far reported [14]. Reconstruction of the haplotypes bearing some of the most common mutations has provided strong evidence that they have either a single or a few common ancestors and may have been present in the population already for several centuries[17-19]. Consequently, the incidence of specific mutations is strongly dependent on the population from which the breast cancer families were ascertained. Thus the

185delAG mutation was picked up mainly in families of Ashkenazi-Jewish origin [20].

The extent of the founder-effect was highlighted by the finding that approximately 1% of all Ashkenazi Jews (i.e. regardless of a positive breast cancer family history) are carrying this mutation [21,22], 8 times that of the incidence of all mutations together in the general population [10]. Specific mutations have also been recurrently detected in breast cancer families of Swedish, British, Italian, and Austrian origin [18,23-26].

Despite the vast number of BRCA1 gene changes detected to date, there remains a discrepancy between the proportion of BRCA1 mutations predicted by linkage studies [6,8], and the actual prevalence established by mutation analysis, among breast cancer families derived from a variety of ethnic backgrounds [27-31]. In general, this is explained in two ways: either a substantial number of mutations have been missed by the applied mutation screening methodology, or the genetic heterogeneity of hereditary breast cancer is significantly greater than hitherto expected.

Relatively little information of predictive value can be gleaned from the existing data. In one set of 35 kindreds with proven BRCA1 mutations from the United Kingdom, the ovarian cancer risk heterogeneity as predicted from linkage studies could be confirmed [25]. Mutations occurring before codon 1435 conferred a significantly higher ovarian cancer risk than those occurring after this point. While this is consistent with earlier predictions based on linkage analysis [9], the current mutation distribution is at odds with the predicted lower frequency of these alleles. In addition, the expressivity of BRCA1 displays considerable inter-family variability. For example, the 185delAG mutation was detected in families with early-onset breast cancer and ovarian cancer, or late-onset breast cancer without ovarian cancer [32]. Clearly, other factors influence the expression of the

phenotype, and some of those might be genetic, others environmental. Of note, BRCA1 carriers who have a rare allele at the HRAS1 minisatellite locus were recently shown to be at a 2.8-fold increased risk for ovarian cancer relative to those carriers who had common alleles at HRAS1 [33]. However, a firm establishment of the full spectrum of BRCA1 gene changes in the population is pivotal for a more formal analysis of this matter.

An intriguing feature of BRCA1, and unexpected in the light of Knudson's two-hit inactivation theorem for tumour suppressor genes, is that somatically acquired mutations are extremely rare in ovarian tumours [34-38] and have in fact not yet been detected in 135 breast tumours [39,40]. This might indicate that inactivation of BRCA1 is not selected for during tumorigenesis of the non-inherited form of breast cancer. BRCA1 expression might be critical only during certain stages of tissue development, e.g., during puberty when the breast undergoes its final differentiation into a potential milk-producing gland [39]. However, others have argued that the mechanism of inactivation might be different from that seen in inherited cases [41]. The present invention now reveals that the unusual high concentration of Alu-elements in the BRCA1 gene intronic regions [11] favors the induction of large genomic deletions and inversions in a situation of increased genomic instability although other mechanisms leading to these mutations may also play significant roles. The present invention thus provides a diagnostic test kit (and means and methods) for determining mutations, especially deletions of relatively large stretches of nucleotides in genes associated with hereditary types of cancer, in particular such mutations (deletions of relatively large stretches of nucleotides) in the BRCA1 gene. Such mutations are difficult, if not impossible, to detect by the currently PCR-based approach (if their occurrence or the site thereof is unknown) using genomic DNA as template, which has

been most widely applied to establish the current mutation spectrum of BRCA1.

The present invention thus provides a diagnostic test kit for detecting the presence of or predisposition for e.g. breast cancer, whereby a means is provided for detecting a deletion of a stretch of nucleotides from a BRCA 1 gene in a sample. Now that it is known that such mutations occur, it is within the skill of the art to arrive at means to determine the presence of these mutations, either the ones disclosed herein or similar mutations. Such means may include hybridization of a probe flanking both sides of the deletion, or using two probes on either side of the deletion and amplifying the stretch in between, another way may be lack of hybridization, when using a probe hybridizing to a deleted part, etc. Yet another way may be lack of amplification between one or more sets of primers targeted at or near a deleted region. This already implicates that typically multiplex PCR approaches are very suitable. Also exon-connection PCR is a very suitable approach for use in the present invention. The techniques mentioned above are well known in the art and need no further explanation. Since mutations as disclosed herein may occur in one allele only, quantitative methods are often preferable. It is of course clear that the diagnostic test kit should provide all other necessary means for determining the presence or absence of the mutations, such as buffers, detection means (possibly labels or markers), etc.

A convenient diagnostic test kit according to the invention apart from amplification methods such as PCR, NASBA and the like is a diagnostic test kit whereby the means comprise the necessary elements for southern blotting. The deletions to be detected are typically relatively large stretches of nucleotides, particularly of a size which when subjected to PCR or similar amplification techniques would not be amplified under normal reaction conditions because of their length. Typically the deletion comprises one or more exons of

the BRCA1 gene or a frameshift and/or a termination codon. An exemplified deletion that is a good marker for the predisposition for cancer is the deletion which comprises at least a major part of exon 22.

Another exemplified deletion that is a good marker for the predisposition for cancer is the deletion which comprises at least a major part of nucleotides 1396-1662.

Another exemplified deletion that is a good marker for the predisposition for cancer is the deletion which comprises at least a major part of exons 13-16.

Another exemplified deletion that is a good marker for the predisposition for cancer is the deletion which comprises at least a major part of exon 13.

An exemplified deletion that is a good marker for the predisposition for cancer is the deletion which comprises a stretch of nucleotides between two ALU-elements. This kind of deletion ties in very nicely with a suggested mechanism of the origin of these mutations and the same may also be found in other genes involved in cancer and having many of these elements.

Thus the invention further provides a probe for use in a diagnostic test kit according to invention comprising a nucleic acid sequence which is a fusion of two (complementary sequences of) ALU elements, in particular of the BRCA1 gene. In general the invention thus provides a probe for use in a diagnostic test kit according to the invention, which is a fusion product of two sequences adjacent to the site of a deletion of a stretch of nucleotides.

Also provided is a method for determining the presence in a sample of a nucleic acid derived from a BRCA1 gene having a deletion of a stretch of nucleotides, comprising contacting said sample with at least one probe which alone or together with other means is capable of distinguishing between BRCA1 genes having said deletion and BRCA1 genes not having said deletion, allowing for possible hybridization

between said probe and said nucleic acid and identifying the hybridization product.

Specific embodiments of the invention will be explained in detail below.

Detailed description of the invention.

The present invention in one of its embodiments, which has been described in detail in the experimental part provides a description and detection in human genomic DNA of large genomic deletions in BRCA1. In addition, the invention shows involvement of the Alu-repeat elements, present at high frequency in the intronic regions of BRCA1 [11], in generating a number of these deletions. The invention also contemplates the frequency of these deletions in the Dutch population, and their descendance from a common ancestor.

We have found that the mutation spectrum of BRCA1 as resolved up to this point [13,42] has been biased by PCR-based mutation-screening methods such as SSCP, the protein truncation test (PTT), and direct sequencing, using genomic DNA as template. We describe as examples thereof two large genomic deletions, which are not detected by these approaches, and which together comprise 38% of all BRCA1 mutations found in a sample of 170 Dutch breast cancer families [43,44]. One deletion removes 510 basepairs (bp) including exon 22 (Figure 1) and was found 8 times. The other deletion removes 3835 bp including exon 13 (Figure 2) and was found 4 times.

The haplotypes of the 8 families with the exon 22 deletion were reconstructed by typing 3 intragenic markers (D17S855, D17S1322, D17S1323) and 2 flanking markers (THRA1 and D17S1327). These haplotypes were completely concordant for the intragenic markers in at least 7 families, and the haplotype conservation extended proximally to THRA1, and distally to D17S1327, in at least 5 families, to comprise a genetic region of approximately 2 cM. The haplotypes of the 4 families with the exon 13 deletion were reconstructed in a

similar way. These haplotypes were completely concordant for the intragenic markers in at least 2 families, and the haplotype conservation extended proximally to THRA1, and distally to D17S1327, in all 4 families, to comprise a genetic region of approximately 2 cM.

Molecular characterization of the deletions revealed that the exon 22 deletion starts in intron 21 and ends within the most upstream copy of three head-to-tail arranged Alu-elements in intron 22. A 17-bp imperfect homology to the intron 22 Alu-element was found at the 5' deletion breakpoint (Figure 3). The 3' breakpoint is closely flanked on either side by two 25-bp sequences strongly homologous to the Alu core-sequence implied to stimulate recombination [45].

The exon 13 deletion starts in intron 12 in an Alu-element (112 bp from the 5' end) and ends in intron 13 in a region which shares very high homology to this element (Figure 4). Both the 5' and the 3' breakpoint are closely flanked on either side by sequences strongly homologous to the 26-bp Alu core-sequence implied to stimulate recombination [45].

The current invention facilitates the design of PCR-based strategies (now that the presence of this kind of mutations is known) to identify the heterozygous presence of the deletions in human genomic DNA. Oligonucleotide primers can be designed so to immediately flank the deletion breakpoints, and allow the specific amplification of a deletion-junction fragment as a diagnostic endpoint. Given the size of the deletions, the wildtype BRCA1 genomic sequence would remain refractory to PCR-amplification under most standard reaction conditions. PCR-based diagnosis is an essential requirement to scale up throughput in the screening for these mutations.

The current invention also pertains to the molecular mechanism which may have generated the genomic deletions in the BRCA1 gene, especially since this needs to be viewed in a broader sense in that the same kind of phenomenon may be

picked up in other genes or in the same gene, but not having anything to do with the inheriting kind of cancer.

The current invention thus also pertains to the role of BRCA1 mutations in non-inherited or sporadic breast cancer.

Experimental part.

The exon 22 deletion was revealed by Southern blot analysis of genomic DNA digested with either HindIII or BglII. As probe we used p1424, which contains ~1-kb cDNA-derived segment from exons 14-24. A carrier of the exon 22 deletion shows aberrant bands of 9.3 kb in the HindIII digest and of 6.7 kb in the BglII digest.

The exon 13 deletion was revealed by Southern blot analysis of genomic DNA digested with either HindIII or BglII. As probes we used either p11 or p1424, which contain ~1-kb cDNA-derived segments from exon 11 and exons 14-24, respectively. A carrier of the exon 13 deletion shows an aberrant band of 6.4 kb in the HindIII pattern obtained with probe p1424 and of 14 kb in the BglII pattern obtained with probe p11.

To further characterize these deletions, we used intronic amplimers to obtain PCR-products from genomic DNA, specifically containing the deletion-junction fragment. Amplimers flanking exon 22 generated an aberrant genomic fragment of 1.4 kb in DNA samples carrying the exon 22 deletion, which turned out to contain a 510-bp deletion relative to the wildtype sequence (Figure 3). The deletion affecting exon 22 removes the bases 79505-80014 (510 bp) as listed in the genomic sequence of BRCA1 (Genbank accession nr. L78833). As a result, 74 basepairs, corresponding to exon 22, are missing in the processed mRNA-transcript (bases 79543-79616 in Genbank accession nr. L78833).

Amplimers flanking exon 13 generated an aberrant genomic fragment of 2.7 kb in DNA samples carrying the exon 13 deletion, which turned out to contain a 3835 bp deletion relative to the wildtype sequence (Figure 4). The deletion

affecting exon 13 removes the bases 44514-48348 (3835 basepairs) as listed in the genomic sequence of BRCA1 (Genbank accession nr. L78833). As a result, 172 basepairs, corresponding to exon 13, are missing in the processed mRNA-transcript (nucleotides 46156-46327 in Genbank accession nr. L78833).

We examined 142 breast cancer families in which thusfar no BRCA1 or BRCA2 mutation had been found (refs. 43,44 and our unpublished results) for the presence of the exon 13 and exon 22 deletions. They were found in 4 and 8 families, respectively. Together with previous mutation screening results, using PTT and direct sequencing [44], these deletions thus comprise 12/32 (38%) of all families in which a BRCA1 mutation has been detected to date. Three intragenic and 2 flanking markers were used to reconstruct the disease haplotype for each of the research families carrying either the 510-bp or 3.8-kb deletion. Strong conservation of allele-lengths was observed at the intragenic loci among the haplotypes carrying the same deletion, in agreement with their descent from a common ancestor.

The haplotype in the Dutch population that carries the 510-bp deletion around exon 22 is characterized by a 155-bp allele at the microsatellite marker D17S855 in intron 20, a 122-bp allele at microsatellite marker D17S1322 in intron 19, and a 151-bp allele at microsatellite marker D17S1323 in intron 12. The haplotype in the Dutch population that carries the 3835-bp deletion around exon 13 is characterized by a 151-bp allele at D17S855, a 122-bp allele at D17S1322, and a 151-bp allele at D17S1323 in intron 12. The primer sequences used to detect these alleles are: for D17S1322: Foward (F) 5' CTAGCCTGGGCAACAAACGA 3' and Reverse (R) 5' GCAGGAAGCAGGAATGGAAC 3'; for D17S855: F 5' GGATGGCCTTT TAGAAAGTGG 3' and R 5' ACACAGACTTGTCCTACTGC 3'; for D17S1323: F 5' TAGGAGATGGATTATTGGTG 3' and R 5' AAGCAACTTTGCAAT GAGTG 3'. PCR conditions have been described elsewhere [44].

Detection of the mutations

Isolation of genomic DNA and total RNA from freshly taken blood samples, and preparation of first-strand cDNA by reverse transcription, has been described [43].

cDNA analysis to detect the exon 13 deletion.

Exons 12-24 were amplified from first-strand cDNA products obtained by reverse transcription using the following primers for the first PCR: F 5'TCACAGTGCAGTGAATTGGAAG 3' and R 5' GTAGCCAGGACAGTAGAAGGACTG 3' . The obtained PCR-products were used as template for a second PCR of exons 12-24 using nested primers (F 5' GAAGAAAGAGGAACGGGCTTGG 3' and R 5' GGCCACTTTGTAAGCTCATTC 3'). PCR conditions were as described previously [43]. Five µl of the final PCR products are analysed on a 1% agarose gel.

cDNA analysis to detect the exon 22 deletion.

Exons 12-24 were amplified from first-strand cDNA products obtained by reverse transcription using the following primers for the first PCR: F 5'TCACAGTGCAGTGAATTGGAAG 3' and R 5' GTAGCCAGGACAGTAGAAGGACTG 3' . The obtained PCR-products were used as template for a second PCR of exons 20-24 using nested primers (F 5' AACCACCAAGGTCCAAAGC 3' and R 5' GTAGCCAGGACAGTAGAAGGACTG 3'). PCR conditions were as described previously[43]. Five µl of the final PCR products are analysed on a 1% agarose gel.

Genomic PCR of the 3835-bp deletion spanning exon 13. A PCR reaction of 50 µl contains 200 ng of genomic DNA, 10 pmol primers (F 5' TAGGAGATGGATTATTGGTG 3' and R 5' TAC GTGGGTTCAACTGAAGC 3'), 0.75 Units Amplitaq Taq polymerase (Perkin-Elmer-Cetus), and 5 µl of 10x ITP/BSA buffer (500 mM KCl, 100 mM TRIS-HCl pH 8.4, 25 mM $MgCl_2$, 2 mg/ml BSA, 2 mM dNTPs). This mixture is heated at 94°C for 5 minutes, followed by 35 cycles of PCR (at 94°C for 45 seconds, at 52°C for 1 min. and at 72°C for

2.5 min on a Perkin-Elmer-Cetus DNA thermal Cycler). The PCR is concluded by an incubation at 72°C for 6 minutes. Five µl of the PCR products are analysed on a 1% agarose gel.

Genomic PCR of the 510-bp deletion spanning exon 22. A PCR reaction of 50 µl contains 300 ng of genomic DNA, 10 pmol primers (F 5' TCCCATTGAGAGGTCTTGCT 3' and R 5' ACTGTGCTACTCAAGCACCA 3'), 0.75 U Amplitaq Taq polymerase (Perkin-Elmer-Cetus), 5 µl Optiprime buffer #6 (Stratagene) and 0.1 mM dNTPs. Thermal cycles are as described for the deletion of 3.8 kb. Five µl of the PCR products are analysed on a 1.5% agarose gel.

Southern analysis.

Five µg of genomic DNA is digested with either the restriction endonuclease BglII or HindIII. Agarose gels (0.8%) are run at 30V for 16 hr in TAE buffer (40 mM Tris-HAc pH 8.3, 1 mM EDTA). Procedures for denaturing, and transferring the separated DNA to nylon membranes (Hybond N+, Amersham) have been described [46]. As probes we used PCR-products obtained from a clone containing the complete BRCA1-cDNA, and purified by using the QIAquick PCR Purification Kit from QIAGEN. Probe-11 (p11) derives entirely from exon 11 and was obtained with the primers F 5' GAAAAAAAAGTACAACCAAATGCC and R 5' AGCCCACTTCATTAGTACTGGAAC 3', and probe-1424 (p1424) contains exons 14-24 and was obtained with the primers F 5' TACCCTATAAGCCAGAATCCAGAA 3' and R 5' GGCCACTTTGTAAGC TCATTC 3'. Purified fragments were labelled using the Megaprime DNA labelling System from Amersham according to suppliers protocols. Hybridizations were carried out at 65°C in 125 mM Na2HPO4.2H2O, 7% SDS, 10% PEG-6000, 1 mM EDTA. Final washing was in 45 mM NaCl, 4.5 mM Na-citrate pH 7.0, 0.1% SDS, at 65 ° C for 30 minutes.

Brief description of the drawings

Figure 1. Schematic representation of the genomic deletion spanning exon 22 of BRCA1. The intronic regions are drawn to scale relative to one another, and the exonic region are drawn to scale relative to one another, but not to intronic regions. The positions of the restriction endonucleases HindIII and BglII, used in Southern blot analysis, are indicated. The arrows indicate the presence and orientation of an Alu-element.

Figure 2. Sequence of exon 22 (upper case) and its flanking intron-sequence (lower case). The numbers refer to the genomic sequence of BRCA1 (Genbank accession nr. L78833). Starting and ending positions of the 510-bp deletion are indicated by hooked arrows and affect positions 79505-80014. The first 241 bp of an Alu-element are depicted in italics, and the boxed sequences are imperfect copies (1 and 5 mismatches, respectively) of a common 26-bp core sequence involved in recombinations leading to gene rearrangements in the LDLR gene [45]. A stretch of 17 bp at the 5' junction of the deletion is homologous to a 19-bp stretch 37 bp upstream of the 3' deletion-breakpoint (underlined with arrows).

Figure 3. Schematic representation of the genomic deletion spanning exon 13 of BRCA1. The intronic regions are drawn to scale relative to one another, and the exonic region are drawn to scale relative to one another, but not to intronic regions. The positions of the restriction endonucleases HindIII and BglII, used in Southern blot analysis, are indicated. The arrowheads indicate the presence and orientation of an Alu-element.

Figure 4. Aligned sequences of intronic regions flanking exon 13, and of the deletion-junction fragment (Jnctn). The upper sequence of each alignment corresponds to intron 12

sequences, the lower sequence intron 13 sequences. The numbers refer to the genomic sequence of BRCA1 (Genbank accession nr. L78833). The boxed sequence indicates the 10 bp where the recombination took place that led to the deletion of 3835 bp. The intron 12 sequence depicted here represents the first 180 bp of an Alu-element. The intron 12 region 44481-44551 shares an 85% identity with the intron 13 region 48316-48386. The underlined sequences are imperfect copies of a common 26-bp core sequence involved in recombinations leading to gene rearrangements in the LDLR gene [45].

References

1. Slattery ML, and Kerber RA (1993) A comprehensive evaluation of family history and breast cancer risk: the Utah Population Database. JAMA 270: 1563-1568

2. Lynch, H. T., Marcus, J. M., Watson, P., Conway, T., Fitzsimmons, M. L. & Lynch, J. F. (1989) in Genetic epidemiology of cancer (eds Lynch, H.T. & Hirayama, T.) p. 289-332, CRC Press, Inc, Boca Raton, Florida.

3. Lynch HT, Albano WA, Heieck JJ, Mulcahy GM, Lynch JF, Layton MA, and Danes BS (1984) Genetics, Biomarkers, and the control of breast cancer: a review. Cancer Genet. Cytogenet. 13: 43-92

4. Claus E, Risch N, and Douglas W (1991) Genetic analysis of breast cancer in the cancer and steroid hormone study. Am. J. Hum. Genet. 48: 232-242

5. Hall J, Lee M, Newman B, Morrow J, Anderson L, Huey B, and King M(1990) Linkage of early-onset familial breast cancer to chromosome 17q21. Science 250: 1684-1689

6. Easton D, Bishop D, Ford D, Crockford G, and the Breast Cancer Linkage Consortium (1993) Genetic linkage analysis in familial breast and ovarian cancer: Results from 214 families. Am. J. Hum. Genet. 52: 678-701

7. Ford D, Easton D, Bishop D, Narod S, Goldgar D, and the Breast Cancer Linkage Consortium (1994) Risks of cancer in BRCA1-mutation carriers. Lancet 343: 692-695

8. Narod S, Ford D, Devilee P, Barkardottir R, Lynch H, Smith S, Ponder B, Weber B, Garber J, Birch J, Cornelis R, Kelsell D, Spurr N, Smyth E, Haites N, Sobol H, Bignon Y, Chang-Claude J, Hamann U, Lindblom A, Borg A, Piver M, Gallion H, Struewing J, Whittemore A, Tonin P, Goldgar D, Easton D, and the Breast Cancer Linkage Consortium (1995) An evaluation of genetic heterogeneity in 145 breast-ovarian cancer families. Am J Hum Genet 56: 254-264

9. Easton D, Ford D, Bishop D, and the Breast Cancer Linkage Consortium (1995) Breast and ovarian cancer incidence in BRCA1-mutation carriers. Am. J. Hum. Genet. 56: 265-271

10. Ford D, Easton D, and Peto J (1995) Estimates of the gene frequency of BRCA1 and its contribution to breast and ovarian cancer incidence. Am. J. Hum. Genet. 57: 1457-1462

11. Smith TM, Lee MK, Szabo CI, Jerome N, Mceuen M, Taylor M, Hood L, and King MC (1996) Complete genomic sequence and analysis of 117 kb of human DNA containing the gene BRCA1. Genome Research 6: 1029-1049

12. Miki Y, Swensen J, Shattuck-Eidens D, Futreal P, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett L, Ding W, Bell R, Rosenthal J, Hussey C, Tran T, McClure M, Frye C, Hattier T, Phelps R, Haugen-Strano A, Katcher H, Yakumo K, Gholami Z, Shaffer D, Stone S, Bayer S, Wray C, Bogden R, Dayananth P, Ward J, Tonin P, Narod S, Bristow P, Norris F, Meera Khan P, Morrison P, Rösteck P, Lai M, Barrett J, Lewis C, Neuhausen S, Cannon-Albright L, Goldgar R, Kamb A, Wiseman R, and Skolnick M (1994) A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 266: 66-71

13. Breast Cancer Information Core (1997) http://www.nhgri.nih.gov/Intramural_research/Lab_transfer/Bic/

14. Couch FJ, Weber BL, Borresen AL, Brody L, Casey G, Devilee P, Fitzgerald M, Friend S, Gayther S, Goldgar D, Murphy P, Szabo C, Weber B, Wiseman R, Anderson T, Durocher F, Ganguly A, King MC, Lenoir G, Narod S, Olopade O, Plummer S, Ponder B, Serova O, Simard J, Stratton M, and Warren B (1996) Mutations and polymorphisms in the familial early onset breast cancer (BRCA1) gene. Hum Mutat 8: 8-18

15. Cornelis R, Neuhausen SL, Johansson O, Arason A, Kelsell D, Ponder BAJ, Tonin P, Hamann U, Lindblom A, Lalle P, Longy M, Olh E, Scherneck S, Bignon Y, Sobol H, Chang-Claude J, Larsson C, Spurr N, Borg A, Barkardottir RB, Narod S, Devilee

P, and the Breast Cancer Linkage Consortium (1995) High allele loss rates at 17q12-q21 in breast and ovarian tumors from BRCA1-linked families. Genes Chrom. Cancer 13: 203-210

16. Sharan S, Wims M, and Bradley A (1995) Murine Brca1: Sequence and significance for human missense mutations. Hum. Mol. Genet. 4: 2275-2278

17. Simard J, Tonin P, Durocher F, Morgan K, Rommens J, Gingras S, Samson C, Leblanc J, Belanger C, Dion F, Liu Q, Skolnick M, Goldgar D, Shattuck-Eidens D, Labrie F, and Narod S (1994) Common origins of BRCA1 mutations in Canadian breast and ovarian cancer families. Nature Genet. 8: 392-398

18. Johannsson O, Ostermeyer EA, Hkansson S, Friedman LS, Johansson U, Sellberg C, Brondum-Nielsen K, Sele V, Olsson H, King MC, and Borg (1996) Founding BRCA1 mutations in hereditary breast and ovarian cancer in southern Sweden. Am. J. Hum. Genet. 58: 441-450

19. Neuhausen SL, Mazoyer S, Friedman L, Stratton M, Offit K, Caligo A, Tomlinson G, Cannon-Albright L, Bishop T, Kelsell D, Solomon E, Weber B, Couch F, Struewing J, Tonin P, Durocher F, Narod S, Skolnick MH, Lenoir G, Serova O, Ponder BAJ, Stoppa-Lyonnet D, Easton D, King M-C, and Goldgar DE (1996) Haplotype and phenotype analysis of six recurrent BRCA1 mutations in 61 families: results of an international study. Am. J. Hum. Genet. 58: 271-280

20. Tonin P, Serova O, Lenoir G, Lynch H, Durocher F, Simard J, Morgan K, and Narod S (1995) BRCA1 mutations in Ashkenazi Jewish women. Am. J. Hum. Genet. 57: 189

21. truewing J, Abeliovich D, Peretz T, Avishai N, Kaback M, Collins F, and Brody L (1995) The carrier frequency of the BRCA1 185delAG mutation is approximately 1 percent in Ashkenazi Jewish individuals. Nature Genet. 11: 198-200

22. oa BB, Boyd AA, Volcik K, and Richards CS (1996) Ashkenazi Jewish population frequencies for common mutations in BRCA1 and BRCA2. Nature Genet. 14: 185-187

23. hattuck-Eidens D, McClure M, Simard J, Labrie F, Narod S, Couch F, Hoskins K, Weber B, Castilla L, Erdos M, Brody L,

Friedman L, Ostermeyer E, Szabo C, King M, Jhanwar S, Offit K, Norton L, Gilewski T, Lubin M, Osborne M, Black D, Boyd M, Steel M, Ingles S, Haile R, Lindblom A, Olsson H, Borg A, Bishop T, Solomon E, Radice P, Spatti G, Gayther S, Ponder B, Warren W, Stratton M, Liu Q, Fujimura F, Lewis C, Skolnick M, and Goldgar D (1995) A collaborative survey of 80 mutations in the BRCA1 breast and ovarian cancer susceptibility gene: Implications for presymptomatic testing and screening. JAMA 273: 535-541

24. agner TMU, Moslinger R, Zielinski C, Scheiner O, and Breiteneder H (1996) New austrian mutation in BRCA1 gene detected in three unrelated HBOC families. Lancet 347: 1263

25. ayther SA, Warren W, Mazoyer S, Russell PA, Harrington PA, Chiano M, Seal S, Hamoudi R, Van Rensburg EJ, Dunning AM, Love R, Evans G, Easton D, Clayton D, Stratton MR, and Ponder BAJ (1995) Germline mutations of the BRCA 1 gene in breast and ovarian cancer families provide evidence for a genotype-phenotype correlation. Nature Genet. 11: 428-433

26. aligo MA, Ghimenti C, Cipollini G, Ricci S, Brunetti I, Marchetti V, Olsen R, Neuhausen S, Shattuckeidens D, Conte PF, Skolnick MH, and Bevilacqua G (1996) BRCA1 germline mutational spectrum in Italian families from Tuscany: a high frequency of novel mutations. Oncogene 13: 1483-1488

27. Castilla L, Couch F, Erdos M, Hoskins K, Calzone K, Garber J, Boyd J, Lubin M, Deshano M, Brody L, Collins F, and Weber B (1994) Mutations in the BRCA1 gene in families with early-onset breast and ovarian cancer. Nature Genet. 8: 387-391

28. Inoue R, Fukutomi T, Ushijima T, Matsumoto Y, Sugimura T, and Nagao M (1995) Germline mutation of BRCA1 in Japanese breast cancer families. Cancer Res. 55: 3521-3524

29. Durocher F, Tonin P, Shattuck-Eidens D, Skolnick M, Narod SA, and Simard J (1996) Mutation analysis of the BRCA1 gene in 23 families with cases of cancer of the breast, ovary, and multiple other sites. Journal Of Medical Genetics 33: 814-819

30. Xu CF, Chambers JA, Nicolai H, Brown MA, Hujeirat Y, Mohammed S, Hodgson S, Kelsell DP, Spurr NK, Bishop DT, and Solomon E (1997) Mutations and alternative splicing of the BRCA1 gene in UK breast/ovarian cancer families. Genes Chromosomes And Cancer 18: 102-110

31. Hamann U, Brauch H, Garvin AM, Bastert G, and Scott RJ (1997) German family study on hereditary breast and/or ovarian cancer: germline mutation analysis of the BRCA1 gene. Genes Chromosomes And Cancer 18: 126-132

32. Friedman L, Szabo C, Ostermeyer E, Dowd P, Butler L, Park T, Lee M, Goode E, Rowell S, and King M (1995) Novel inherited mutations and variable expressivity of BRCA1 alleles, including the founder mutation 185delAG in Ashkenazi Jewish families. Am. J. Hum. Genet. 57: 1284-1297

33. Phelan CM, Rebbeck TR, Weber BL, Devilee P, Ruttledge MH, Lynch HT, Lenoir GM, Stratton MR, Easton DF, Ponder BAJ, Cannon-Albright L, Larsson C, Goldgar DE, and Narod SA (1996) Ovarian cancer risk in BRCA1 carriers is modified by the HRAS1 variable number of tandem repeat (VNTR) locus. Nature Genet. 12: 309-311

34. Hosking L, Trowsdale J, Nicolai H, Solomon E, Foulkes W, Stamp G, Signer E, and Jeffreys A (1995) A somatic BRCA1 mutation in an ovarian tumour. Nature Genet. 9: 343-344

35. Merajver S, Pham T, Caduff R, Chen M, Poy E, Cooney K, Weber B, Collins F, Johnston C, and Frank T (1995) Somatic mutations in the BRCA1 gene in sporadic ovarian tumours. Nature Genet. 9: 439-443

36. Takahashi H, Behbakht K, McGovern P, Chiu H, Couch F, Weber B, Friedman L, King M, Furusato M, LiVolsi, V, Menzin A, Liu P, Benjamin, I, Morgan M, King S, Rebane B, Cardonick A, Mikuta J, Rubin S, and Boyd J (1995) Mutation analysis of the BRCA1 gene in ovarian cancers. Cancer Res. 55: 2998-3002

37. Matsushima M, Kobayashi K, Emi M, Saito H, Saito J, Suzumori K, and Nakamura Y (1995) Mutation analysis of the BRCA1 gene in 76 Japanese ovarian cancer patients: Four

germline mutations, but no evidence of somatic mutation. Hum. Mol. Genet. 4: 1953-1956

38. Lancaster JM, Cochran CJ, Brownlee HA, Evans AC, Berchuck A, Futreal PA, and Wiseman RW (1996) Detection of BRCA1 mutations in women with early onset ovarian cancer by use of the protein truncation test. J. Natl. Cancer Inst. 88: 552-554

39. Futreal P, Liu Q, Shattuck-Eidens D, Cochran C, Harshman K, Tavtigian S, Bennett L, Haugen-Strano A, Swensen J, Miki Y, Eddington K, McClure M, Frye C, Weaver-Feldhaus J, Ding W, Gholami Z, Soederkvist P, Terry L, Jhanwar S, Berchuck A, Iglehart J, Marks J, Ballinger D, Barrett J, Skolnick M, Kamb A, and Wiseman R (1994) BRCA1 mutations in primary breast and ovarian carcinomas. Science 266: 120-122

40. Katagiri T, Emi M, Ito I, Kobayashi K, Yoshimoto M, Iwase T, Kasumi F, Miki Y, Skolnick MH, and Nakamura Y (1996) Mutations in the BRCA1 gene in Japanese breast cancer patients. Hum. Mutat. 7: 334-339

41. Thompson M, Jensen R, Obermiller P, Page D, and Holt J (1995) Decreased expression of BRCA1 accelerates growth and is often present during sporadic breast cancer progression. Nature Genet. 9: 444-450

42. Stratton MR, and Wooster R (1996) Hereditary predisposition to breast cancer. Curr Opin Genet Dev 6: 93-97

43. Hogervorst F, Cornelis R, Bout M, Van Vliet M, Oosterwijk J, Olmer R, Bakker B, Klijn J, Vasen H, Meijers-Heijboer H, Menko F, Cornelisse C, Den Dunnen J, Devilee P, and Van Ommen G (1995) Rapid detection of BRCA1 mutations by the Protein Truncation Test. Nature Genet. 10: 208-212

44. Peelen T, Van Vliet M, Petrij-Bosch A, Mieremet R, Szabo C, Van den Ouwenland AMW, Hogervorst F, Brohet R, Ligtenberg MJL, Teugels E, Van der Luijt R, Van der Hout AH, Gille JJP, Pals G, Jedema I, Olmer R, Van Leeuwen I, Newman B, Plandsoen M, Van der Est M, Brink G, Hageman S, Arts PJW, Bakker MM, Willems HW, Van der Looij E, Neyns B, Bonduelle M, Jansen R, Oosterwijk JC, Sijmons R, Smeets B, Van Asperen CJ, Meijers-

Heijboer H, Klijn JGM, De Greve J, King M-C, Menko FH, Brunner HG, Halley D, Van Ommen G-JB, Vasen HFA, Cornelisse CJ, Van 't Veer LJ, De Knijff P, Bakker E, and Devilee P (1997) A high proportion of novel mutations in BRCA1 with strong founder effects among Dutch and Belgian hereditary breast and ovarian cancer families. Am J Hum Genet 60: 1044-1049

45. Rudiger NS, Gregersen N, and Kielland Brandt MC (1995) One short well conserved region of Alu-sequences is involved in human gene rearrangements and has homology with prokaryotic chi. Nucleic Acids Res 23: 256-260

46. Devilee P, Van den Broek M, Kuipers-Dijkshoorn N, Kolluri R, Meera Khan P, Pearson P, and Cornelisse C (1989) At least four different chromosomal regions are involved in loss of heterozygosity in human breast carcinoma. Genomics 5: 554-560

CLAIMS

1. A diagnostic test kit for detecting the presence of or predisposition for breast cancer, whereby a means is provided for detecting a deletion of a stretch of nucleotides from a BRCA 1 gene in a sample.

2. A diagnostic test kit according to claim 1 whereby the means comprises at least one probe for hybridization.

3. A diagnostic test kit according to claim 2 whereby the means comprise the necessary elements for Southern blotting.

4. A diagnostic test kit according to claim 2 or 3 whereby the probe comprises a sequence complementary to sequences on both sides of the deletion in the BRCA 1 gene .

5. A diagnostic test kit according to anyone of the aforegoing claims whereby the deletion comprises one or more exons of the BRCA1 gene.

6. A diagnostic test kit according to anyone of the aforegoing claims whereby the deletion comprises a frameshift and/or a termination codon.

7. A diagnostic test kit according to anyone of the aforegoing claims whereby the deletion comprises at least a major part of exon 22.

8. A diagnostic test kit according to anyone of the aforegoing claims whereby the deletion comprises a major part of nucleotides 1396-1662.

9. A diagnostic test kit according to anyone of the aforegoing claims whereby the deletion comprises at least a major part of exons 13-16.

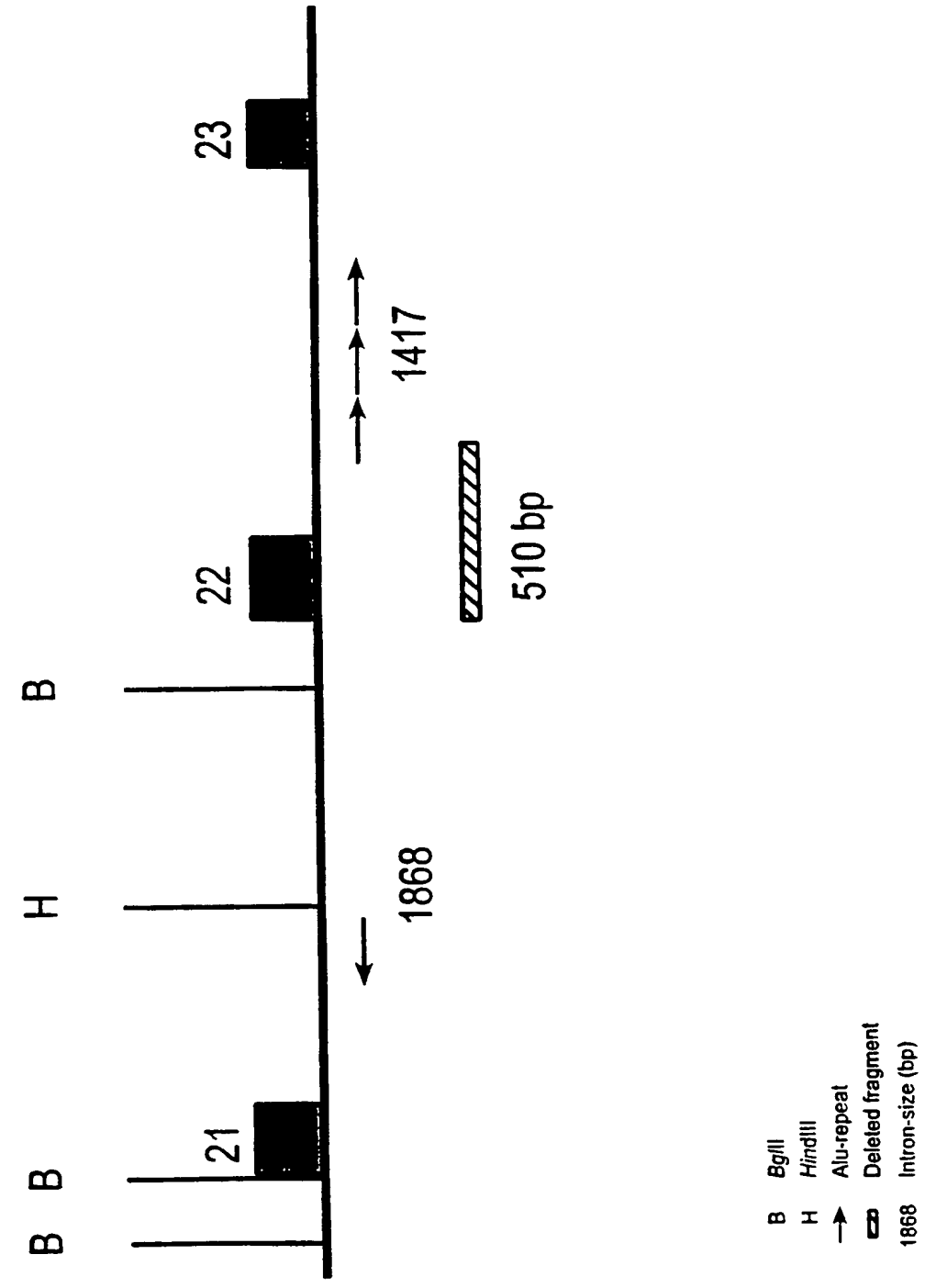
10. A diagnostic test kit according to anyone of the aforegoing claims whereby the deletion comprises at least a major part of exon 13.

11. A diagnostic test kit according to anyone of the aforegoing claims whereby the deletion comprises a deletion of a stretch of nucleotides between two ALU-elements.

12. A probe for use in a diagnostic test kit according to anyone of the aforegoing claims comprising a nucleic acid sequence which is a fusion of two ALU elements of the BRCA1 gene.

13. A probe for use in a diagnostic test kit according to anyone of claims 1-11, which is a fusion product of two sequences adjacent to the site of a deletion of a stretch of nucleotides.

14. A method for determining the presence in a sample of a nucleic acid derived from a BRCA1 gene having a deletion of a stretch of nucleotides, comprising contacting said sample with at least one probe which alone or together with other means is capable of distinguishing between BRCA1 genes having said deletion and BRCA1 genes not having said deletion, allowing for possible hybridization between said probe and said nucleic acid and identifying the hybridization product.


B B H B
21
22
23
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1417
510 bp
B BglII
H HindIII
Alu-repeat
Deleted fragment
1868 Intron-size (bp)

Figure 1

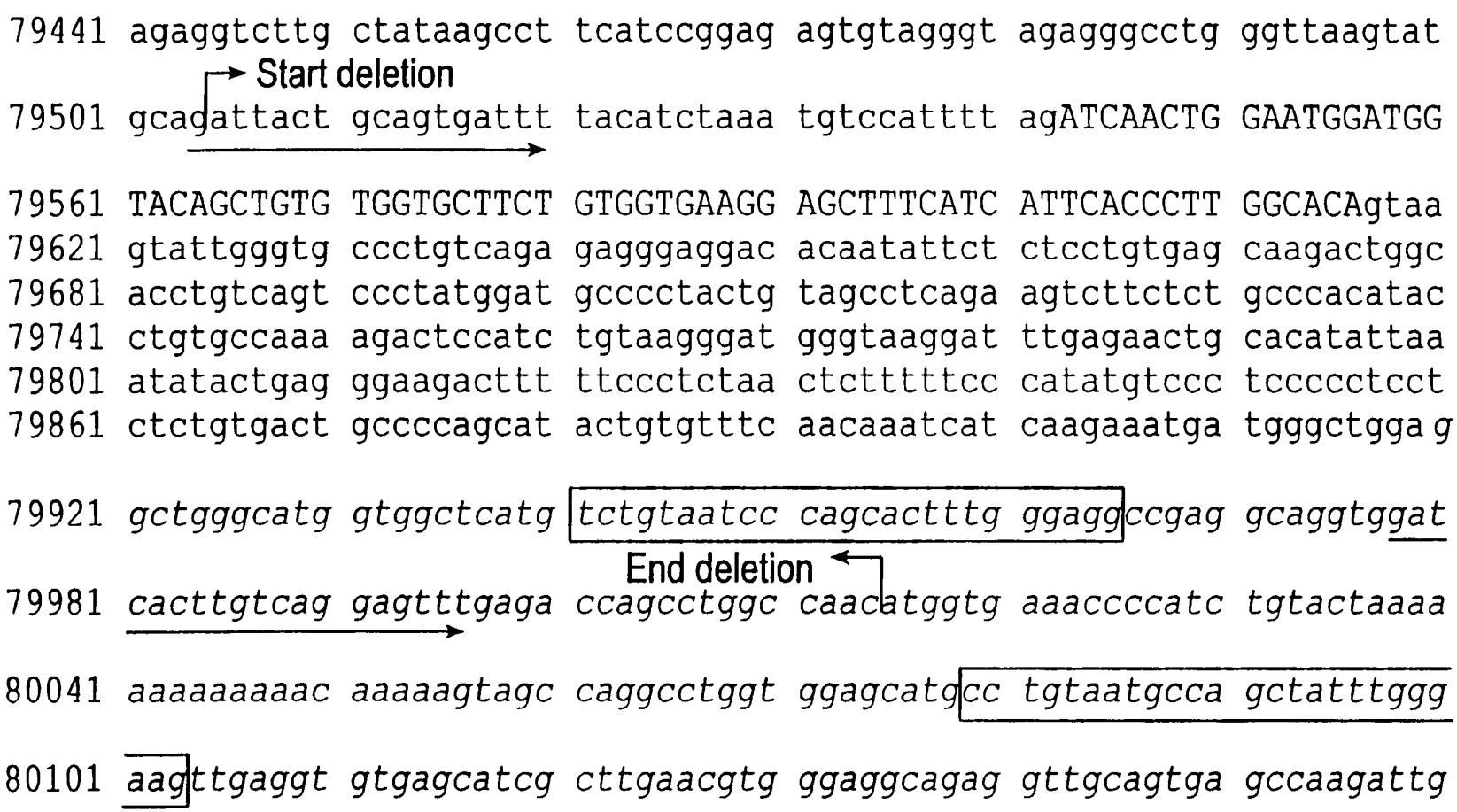
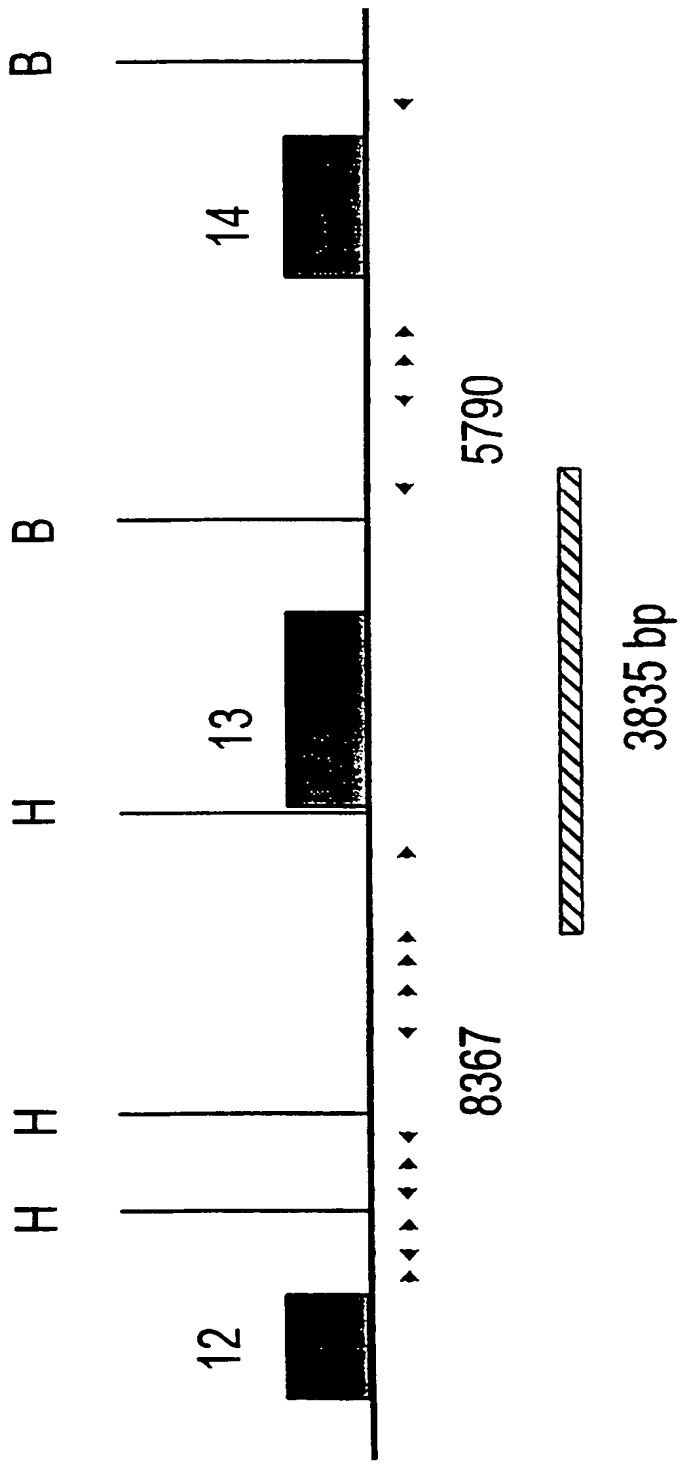


Figure 2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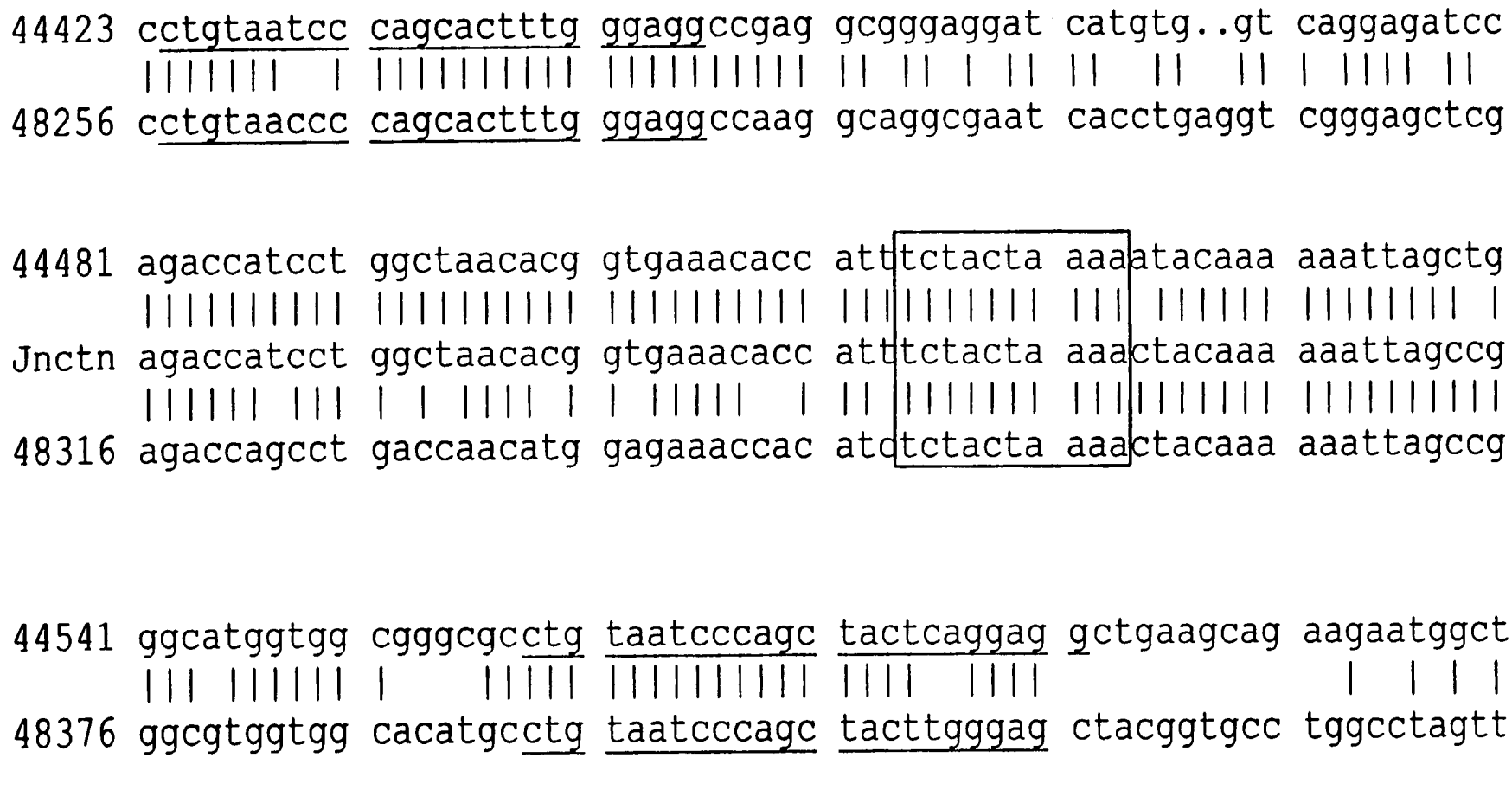
Alu-repeat
Deleted fragment
5790 Intron-size (bp)

Figure 3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44481 agaccatcct ggctaacacg gtgaaacacc atttctacta aaaatacaaa aaattagctg
Jnctn agaccatcct ggctaacacg gtgaaacacc atttctacta aaactacaaa aaattagccg
48316 agaccagcct gaccaacatg gagaaaccac atctctacta aaactacaaa aaattagccg
44541 ggcatggtgg cgggcgcctg taatcccagc tactcaggag gctgaagcag aagaatggct
48376 ggcgtggtgg cacatgcctg taatcccagc tacttgggag ctacggtgcc tggcctagtt

Figure 4

INTERNATIONAL SEARCH REPORT

Interna al Application No
PCT/NL 98/00325

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12Q1/68

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12Q

Documentation searched other than minimumdocumentation to the extent that such documents are included in the fields searcned

Electronic data base consulted during the international search (name of data base and. where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication. where appropriate, of the relevant passages	Relevant to claim No.
X	PUGET ET AL.: "A 1-kb Alu mediated germ line deletion removing BRCA1 exon 17" CANCER RESEARCH., vol. 57, March 1997, pages 828-831, XP002057724 MD US see the whole document ---	1-6, 11-14
X	EP 0 705 903 A (MYRIAD GENETICS INC ;RECH DU CHUL CENTRE (CA); CANCER INST (JP)) 10 April 1996 see the whole document ---	1-6,14
X	US 5 622 829 A (KING MARY-CLAIRE ET AL) 22 April 1997 see column 4, line 15 - line 30; table 1 ---	1-6,14
	-/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of theinternational search	Date of mailing of the international search report
23 September 1998	07/10/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Molina Galan, E

1

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	EP 0 699 754 A (UNIV UTAH RES FOUND ;US GOVERNMENT (US); MYRIAD GENETICS INC (US)) 6 March 1996 see claims; example 9; table 11 ---	1-3,6,14
X	WO 94 21791 A (BERGMANN JOHANNA EUGENIE ;PREDDIE RICK ENRIQUE (CA)) 29 September 1994 see page 15 ---	1
A	SMITH ET AL.: "Complete genomic sequence and analysis of 117 kb of human DNA containing the gene BRCA1" GENOME RESEARCH., vol. 6, 1996, pages 1029-1049, XP002057725 ING HARBOR LABORATORY PRESS US cited in the application see the whole document ---	1-14
A	COUCH ET AL.: "Mutations and polymorphisms in the familial early onset breast cancer (BRCA1) gene" HUMAN MUTATION, vol. 8, 1996, pages 8-18, XP002057726 cited in the application see the whole document ---	1-14
A	RÜDIGER: "One short well conserved region of Alu sequences is involved in human rearrangements and has homology with prokaryotic chi" NUCLEIC ACIDS RESEARCH, vol. 23, no. 2, 1996, pages 256-260, XP002057727 OXFORD GB cited in the application see the whole document ---	11,12
P,X	DATABASE MEDLINE US NATIONAL LIBRARY OF MEDICINE (NLM), BETHESDA, MD, US PETRIJ-BOSCH A ET AL: "BRCA1 genomic deletions are major founder mutations in Dutch breast cancer patients 'published erratum appears in Nat Genet 1997 Dec;17(4):503!." XP002078428 see abstract & NATURE GENETICS, (1997 NOV) 17 (3) 341-5. JOURNAL CODE: BRO. ISSN: 1061-4036., United States --- -/--	1-14

INTERNATIONAL SEARCH REPORT

Intern ial Application No
PCT/NL 98/00325

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication.where appropriate, of the relevant passages	Relevant to claim No.
P,X	US 5 756 294 A (WHITE MARGA B ET AL) 26 May 1998 see the whole document	1-6

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Interr. ial Application No
PCT/NL 98/00325

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0705903 A	10-04-1996	US 5693473 A	02-12-1997
		US 5709999 A	20-01-1998
		AU 691958 B	28-05-1998
		AU 3242895 A	07-03-1996
		AU 686004 B	29-01-1998
		AU 3321295 A	07-03-1996
		AU 691331 B	14-05-1998
		AU 3321695 A	07-03-1996
		CA 2196790 A	22-02-1996
		CA 2196795 A	22-02-1996
		CA 2196797 A	22-02-1996
		CN 1159829 A	17-09-1997
		CN 1172502 A	04-02-1998
		EP 0705902 A	10-04-1996
		EP 0699754 A	06-03-1996
		FI 970513 A	07-04-1997
		FI 970514 A	07-04-1997
		FI 970515 A	07-04-1997
		JP 10505742 T	09-06-1998
		NO 970624 A	14-04-1997
		NO 970625 A	14-04-1997
		NO 970626 A	14-04-1997
		WO 9605306 A	22-02-1996
		WO 9605307 A	22-02-1996
		WO 9605308 A	22-02-1996
		US 5747282 A	05-05-1998
		US 5710001 A	20-01-1998
		US 5753441 A	19-05-1998
US 5622829 A	22-04-1997	AU 5566896 A	07-11-1996
		CA 2217668 A	24-10-1996
		EP 0821733 A	04-02-1998
		WO 9633271 A	24-10-1996
EP 0699754 A	06-03-1996	US 5747282 A	05-05-1998
		US 5710001 A	20-01-1998
		AU 691958 B	28-05-1998
		AU 3242895 A	07-03-1996
		AU 686004 B	29-01-1998
		AU 3321295 A	07-03-1996

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern ial Application No
PCT/NL 98/00325

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP 0699754 A		AU 691331 B	14-05-1998
		AU 3321695 A	07-03-1996
		CA 2196790 A	22-02-1996
		CA 2196795 A	22-02-1996
		CA 2196797 A	22-02-1996
		CN 1159829 A	17-09-1997
		CN 1172502 A	04-02-1998
		EP 0705902 A	10-04-1996
		EP 0705903 A	10-04-1996
		FI 970513 A	07-04-1997
		FI 970514 A	07-04-1997
		FI 970515 A	07-04-1997
		JP 10505742 T	09-06-1998
		NO 970624 A	14-04-1997
		NO 970625 A	14-04-1997
		NO 970626 A	14-04-1997
		WO 9605306 A	22-02-1996
		WO 9605307 A	22-02-1996
		WO 9605308 A	22-02-1996
		US 5693473 A	02-12-1997
		US 5709999 A	20-01-1998
		US 5753441 A	19-05-1998
WO 9421791 A	29-09-1994	NONE	
US 5756294 A	26-05-1998	NONE	